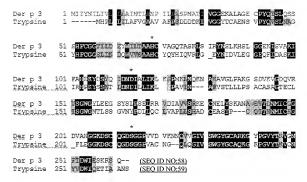
Amendments to the Specification:

Please replace the paragraph on page 7, beginning line 12, with the following amended paragraph:

The Sequence homology between Der p 3 and bovine trypsin are shown below. Residues signalled with * are implicated in the catalytic site.



Please replace the paragraph on page 13, beginning line 11, with the following amended paragraph:

A further aspect of the present invention provides an isolated nucleic acid encoding a mutated version of the Der p 1/ProPor p 1/ProPorDer p 1 allergen as disclosed herein. Preferably the nucleotide sequence is a DNA sequence and can be synthesized by standard DNA synthesis techniques, such as by enzymatic ligation as described by D.M. Roberts et al in Biochemistry 1985, 24, 5090-5098, by chemical synthesis, by in vitro enzymatic polymerization, or by a combination of these techniques. Preferably the nucleic acid sequence has a codon usage pattern that has been optimised so as to mimic the one used in the intended expression host, more preferably resembling that of highly expressed mammalian e.g. human genes. Preferred DNA sequences are codon-optimised sequences and are set out in SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:10, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:14, SEQ ID NO:15 and SEQ ID NO:18.

Please replace the paragraph on page 35, beginning line 4, with the following amended paragraph:

2. - Site-directed mutagenesis

65 to arginine (C65R).

Mutagenesis of Der p 1 cysteine residues at position 4, 31 or 65 (mature ProDer p 1 numbering, corresponds to positions 84, 111 or 145 in ProDer p 1) was performed in the plasmid pNIV4854, after the substitution of DNA fragments carrying one of the three cysteine codons by synthetic oligonucleotides containing the mutations. The following oligonucleotides were used:

5'TTAAGACCCAGTTTGATCTCAACGCGGAGACCAACGCCCGTATCAACGGCAAT GCCCCGCTGAGATTGATCTGCGCCAGATGAGGACCGTGACTCCCATCCGCATGC (forward) (SEO ID NO:27) and 5'CGGATGGGAGTCACGGTCCTCATCTG GCGCAGATCAATCTCAGCGGGGGCATTGCCGTTGATACTACGGGCGTTGGTCTCC GCGTTGAGATCGAAACTGGGTC3' (reverse) (SEO ID NO:28) to generate a 110bp Afl II-Sph I fragment for the mutation of cysteine residue 4 to arginine (C4R), 5'CAAGGCGGCCGTGGGTCTTGTTGGGCCTTTTCAGGCGTGGCCGCGACAGAGTC GGCATACCTCGCGTATCGGAATCAGAGCCTGGACCTCGC3' (forward) (SEO ID NO:29) and 5'TCAGCGAGGTCCAGGCTCTGATTCCGATACGCGAGGTATGCCGACTCTGTCGCG GCCACGCCTGAAAAGGCCCAACAAGACCCACGGCCGCCTTGCATG3' (reverse) (SEO ID NO:30) to generate a 98bp Sph I-Blp I fragment for the mutation of cysteine residue 31 arginine (C31R). 5'TGAGCAGGAGCTCGTTGACCGTGCCTCC to ${\tt CAACACGGATGTCATGGGGATACGATTCCCAGAGGTATCGAATACATCCAGCAT}$ A3' (forward) (SEO ID NO:31) and 5'CTGGATGTATTCGATACCTCTGGGAATCGTAT CC CCCATGACATCCGTGTTGGGAGGCACGGTCAACGCGCTCCTGC3' (reverse) (SEO ID NO:32) to generate a 82bp Afl II-Sph I fragment for the mutation of cysteine residue

Please replace the paragraph on page 39, beginning line 30, with the following amended paragraph:

Primer1: 5'-GCTATTACCGATACGTAGGC-3' (SEQ ID NO:50)

This primer comprises the Snabl restriction site downstream of the zone to be deleted.

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Please replace the paragraphs on page 40, beginning line 30, with the following amended paragraphs:

Primer2:5'-CCGTTGTCGCGATCCTTGATTCCGATGATGACAGCG-3' (SEQ ID NO:51)

This primer is therefore homologous to part of the ProDer p 1 sequence, that downstream and upstream of the zone to be deleted.

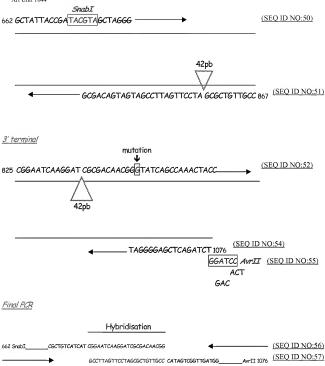
Primer3:

5'-CGGAATCAAGGATCGCGACAACGGGTATCAGCCAAACTACC-3' (SEQ ID NO:52)

This primer is also homologous to part of the ProDer p 1 sequence and will also allow deletion of 42pb. In addition, it contains a point mutation which will make it possible to modify the EcoRV site.

Primer4: 5'-TAGGGGAGCTCAGATCTGATCCACTGAC-3' (SEQ ID NO:53)

Please replace the paragraphs on page 41, beginning line 1, with the following amended paragraphs:



Please replace the paragraphs on page 42, beginning line 23, with the following amended paragraphs:

1. Construction of a PreProDer p 3 synthetic cDNA

A PreProDer p 3 cDNA was synthesised using a set of 10 partially overlapping oligonucleotides. These primers were designed, based on the codon preference of highly expressed E. Coli bacterial genes, and produced by a 394 DNA/RNA Applied Biosystem synthetizer. The degenerately encoded amino acids were not encoded by the most prevalent codons but taking the frequencies of the individual codons into account. For example, AAG or AAA encodes the lysine residue with a respective frequency of 21.45% and 78.55% in highly expressed E. Coli bacterial genes. Consequently, we attempted to follow the same codon frequency instead of selecting only the AAA codon for each lysine residue in the synthetic PreProDer p 3. The oligonucleotides were the following:

5'TCATGATCATCTACAACATTCTGATCGTACTCCTGCTGGCCATTAACACTTTGGC
TAATCCGATCCTGCCGGCATCCCCGAACGCGACCATCGTTGGC 3' (oligo 1, coding) (SEQ ID NO:34)

5'CACCACAGAAGTGGCTACTAGACTGCAGGGAGATCTGATATGGGCACTCACCA GCCAGTGCTTTTTCGCCGCCAACGATGGTCGCG 3' (oligo 2, noncoding) (SEQ ID NO:35)

5'GTAGCCACTTCTGTGGTGGTACTATTCTTGACGAATACTGGATCCTGACCGC GGCACACTGCGTGGCCGGCCAAACAGCGAGCAAACTCTCC 3' (oligo 3, coding) (SEQ ID NO:36)

5'GTCGATCTGGTAGCTATCATATTTTTCATGTGCGAAAATTTTAGCAACAGAGAT CTTTTCGCCACCCAGTGAGTGTTTCAGGCTGTTGTAACGAATGGAGAGTTTGCTC GCTG 3' (oligo 4, noncoding) (SEQ ID NO:37)

5'GATAGCTACCAGATCGACAATGACATTGCGCTGATCAAGCTGAAATCCCCTAT GAAGCTGAACCAGAAAAACGCCAAAGCTGTGGGCCTGC 3' (oligo 5, coding) (SEQ ID NO:38) USSN 10/547,206 Art Unit 1644

5'CTACTCCCTGCCGTCTGAATTACGCCGTGTTGATATCGCTGTGGTATCTCGCAA AGAATGTAACGAGCTGTACTCGAAAGCGAACGCTGAAGTCAC 3' (oligo 7, coding) (SEO ID NO:40)

5'CCACCAGAATCGCCTTGACAAGAGTCCTTACCGCCGTTCGCAACATCACCACCG CAGATCATATTGTCGGTGACTTCAGCGTTCGC 3' (oligo 8, noncoding) (SEQ_ID_NO:41)

5'CAAGGCGATTCTGGTGGGCCGGTGGTCGACGTTAAAAACAACCAGGTTGTAGG TATCGTTTCCTGGGGCTACGGTTGCGCACGTAAAGGC3' (oligo 9, coding) (SEO ID NO:42)

5'AAGCTTTCAGTGGTGGTGGTGGTGGTGCTGCTACGTTTAGATTCAATCCAATC GATAAAGTTACCAACGCGCGTGTACACACCCGGATAGCCTTTACGTGCGCAAC 3' (oligo 10, noncoding). (SEQ ID NO:43)

Please replace the paragraphs on page 44, beginning line 15, with the following amended paragraphs:

Intra recPreProDer p 3 oligonucleotides were used to sequence the insert:

5'AAGCTGAAATCCCCTATGAAGC3' (coding) (SEQ ID NO:44) 5'CTCTTCCAGATAACCCCAGCC3' (noncoding). (SEQ ID NO:45)

Only clone 3 proved to be correct, but missing the first 6 bases on 5' coding end. The addition of the missing bases was achieved by the use of two new oligonucleotides:

5'TTTTATTCATGATCATCTACAACATTCTGATCC3' (oligo 11, coding) (SEQ ID NO:46)

5'GATGCATGCTCGAGCGGC3' (oligo 12, noncoding) (SEQ ID NO:47).

Please replace the paragraph on page 45, beginning line 5, with the following amended paragraph:

The deletion of the putative Der p 3 signal peptide was performed by PCR and using two new primers: 5'CATATGAATCCGATCCTGCCGGCATCCCC3' (oligo 13, coding) (SEQ_ID_NO.48) and 5'GGATCCTCACTGGCTACGTTTAGATTCAATCC3' (oligo 14, non coding) (SEQ_ID_NO.49) Amplification of the ProDer p 3 cDNA was done by PCR with Taq Polymerase (Roche Diagnostics), 15 cycles: denaturation at 97°C for 30 s, annealing at 65°C for 30 s and elongation at 72°C for 1 min. The resulting 750bp fragment was cloned into a pCRII-TOPO eloning vector (Invitrogen). Top 10 competent E Coli were transformed by the resulting plasmid. 9 clones appeared positively inserted; digestion with *Eco*RI proved clones 1,4,8 to be correctly inserted, while sequencing showed that only clone 4 had the right sequence. The ProDer p 3 cDNA was isolated after the digestions with *Nde*I and *XhoI* and cloned into pET15b digested by the same enzymes. The BL21 and BL21 Star E Coli (Invitrogen) strains were transformed by the resulting plasmid Addition of IPTG in the culture medium induced the expression of recombinant ProDer p 3 carrying (His)₆ tag at its N-terminal end.

Please replace the paragraph on page 62, beginning line 26, with the following amended paragraph:

The ProDer p 1 coding cassette from pNIV4846 (full-length 1-302aa ProDer p 1 cDNA with optimised mammalian codon usage) was amplified by PCR using the following primers: 5'ACTGACAGGCCTCGGCCGAGCTCCATTAA3' (Stu1 restriction site in bold, forward) (SEQ ID NO:33) and 5'CAGTCACCTAGGTCTAGACTC GAGGGGAT3' (AvrII restriction site in bold, reverse) (SEQ ID NO:22). The amplified fragment was cloned into the pCR2.1 TOPO cloning vector. The correct ProDer p 1 cassette was verified by DNA sequencing. Recombinant TOPO vector was digested with Stu1-AvrII to generate a 918bp fragment which was introduced into the pPIC9K expression vector restricted with SnaB1-

AvrII. The resulting plasmid, pNIV4878, contains the ProDer p 1 cassette downstream to the Scerevisae α factor

Please replace the paragraphs on page 63, beginning line 6, with the following amended paragraph:

Expression plasmid for the production of unglycosylated ProDer p 1 (N52Q, mature Der p 1 numbering) was derived from pNIV4878 by overlap extension PCR using a set of four primers. The following primers:

5'GGCTTTCGAACACCTTAAGACCCAG3' (primer 1, AffII restriction site in bold, forward) (SEO ID NO:23) and 5'GCTCCCTAGCTACGTA TCGGTAATAGC3' (primer 2, SnaBI restriction site in bold, reverse) (SEO ID NO:24) were used to amplify a 317bp fragment encoding the ProDer p 1 amino acid sequence 71-176.

The following primers 5'CCTCGCGTATCGGCAACAGAGCCTGGACC3' (primer 3, mutation N52Q in bold, forward) (SEQ ID NO:25) and 5'GGTCCAGGCTCTGTTGCC GATACGCGAGG3' (primer 4, mutation N52Q in bold, reverse) (SEQ ID NO:26) were used to introduce mutation N52Q in the ProDer p 1 sequence.